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Communication

Chromatographic NMR in NMR solvents

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ABSTRACT

Recently, it was demonstrated that pseudo-chromatographic NMR experiments could be performed using typical chromatographic solids and solvents. This first setup yielded improved separation of the spectral components of the NMR spectra of mixtures using PFG self-diffusion measurements. The method (dubbed Chromatographic NMR) was successively shown to possess, in favorable cases, superior resolving power on non-functionalized silica, compared to its LC counterpart. To further investigate the applicability of the method, we studied here the feasibility of Chromatographic NMR in common deuterated solvents. Two examples are provided, using deuterated chloroform and water, for homologous compounds soluble in these solvents, namely aromatic molecules and alcohols, respectively.

1. Introduction

Separation techniques are a pivotal element in the analysis of mixtures, as they reduce the complexity of the investigation to a sum of elementary characterization. Chromatography achieves progressive physical separation of the analytes in a mixture by selectively delaying their evolution toward the end of the specific chromatographic tool (a column, a plate, etc.). The nature of the compounds separated can be revealed by any suitable detector. Among those, NMR, along with mass spectrometry, are certainly the most useful ones when unknown molecules have to be revealed. While a complete and satisfactory description of the active mechanisms in chromatography has not been found, the generic model involves chemical exchange between mobile and immobilized phases. Separation occurs if two molecules have sufficient difference in relative translational motion to travel along the chromatographic media, with respect to the length of this latter.

Recently, we have proposed an alternative technique, NMR based, which allows a simplified analysis of mixtures by copycatting this basic principle [1]. A selective delaying of the molecular components of the sample is induced by addition of a suitable solid phase. The corresponding increased spread in the molecular translational diffusion facilitates the utilization of standard PFG-based techniques to decompose the NMR spectrum into the ones of the pure components. The method thus capitalized on the intrinsic interest of PFG-based experiments in mixture analysis, by easing the associated notorious limits in resolution, which have been the focus of improved data processing ([2, and references therein]) or of elaborate multidimensional designs [3, and references therein]. The induced heterogeneity of the mixture calls for moderate spinning rate HRMAS for recovering highly resolved spectra. Nonetheless, faithful apparent diffusion measurements can be performed even in these conditions [4].

We discovered furthermore that, in favorable cases, Chromatographic NMR (Chr NMR) could achieve spectral separation in conditions in which LC produces coelution [5]. More specifically, bare silica was shown to be able to successfully separate the spectra of a mixture of aromatic homologue molecules dissolved in an organic solvent/water mix, a problem typically requiring reverse phase high-performance liquid chromatography (RPLC) and related materials. Although the precise rationale behind this unexpected difference is currently under investigation, preliminary results highlight as a likely source of the enhanced performance a more pronounced role of the solid phase in the NMR experiments. It would thus appear that, in these conditions, the main contribution of the solvent would be just to dissolve an adequate amount of molecules of the analytes, rather than participating in the partition/equilibrium process. If this hypothesis holds true, some of the constraints on the mobile phase composition typical of LC can be safely lifted, and a wider selection of solvent compositions explored.

In this respect, to further integrate Chr NMR into a standard set of analytical tools, it is worth assessing the potential of typical NMR solvents within this framework. Note that, other than the useful property of being available in deuterated form, these

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compounds have been selected over the years for being capable of solubilizing promptly most bioorganic molecules. For instance, anthracene is scarcely soluble in a water/acetonitrile mixture, but very soluble in chloroform. Such an increased solubility is a major advantage, as the sensibility limitation of NMR requires the use of concentrated solutions, about two orders of magnitude more so than for other common LC detectors.

Aim of this work is thus to explore the possibility that common deuterated solvents can effectively serve as the "mobile phases" in chromatographic NMR. As a proof of principle, and inspired by typical chromatographic tests, we analyzed two mixtures of homologues molecules, respectively, soluble in an organic solvent (chloroform in this case) or in water. In fact, the measurement of the behavior of homologue molecules is particularly useful to detect the source of the analyte/solid interaction, which should become stronger increasing the number or strength of the interacting unit.

2. Results and discussion

The NMR diffusivity charts (DOSY plots) for the test sets are shown in Figs. 1 and 2, depicting a 2D correlation between the ^{1}H NMR spectrum and the associated apparent self-diffusion coefficient, D.

They were obtained assuming the theoretical equation linking the signal intensity decay in a stimulated echo experiment while increasing the strength of the PFG, g, applied for a duration δ, with a time Δ allocated to free diffusion of the molecule before echo formation [6]

$$I(g) = I(0)\exp\left[-D(\gamma\delta g)^2(\Delta - \delta/3)\right] \quad (1)$$

If during the time Δ the molecule is accessing areas of the sample in which its self-diffusion varies, a multimodal behavior is imposed onto the intensity decay.

A particular case of a heterogeneous mobility environment is the one under scrutiny here. In the most simplified model, a free and an adsorbed state can be envisioned for a molecule in the presence of the silica gel. The most viable case for a simplified interpretation is when a fast equilibration is achieved among all of the diffusion states, which results in an average apparent diffusion constant. This effect can be expressed, in a first approximation, as an average apparent diffusion coefficient

$$\langle D\rangle = x_{\text{free}} D_{\text{free}} + x_{\text{bound}} D_{\text{bound}} \quad (2)$$

This relation can be extended if a series of equilibria between several heterogeneous environments. Since $D_{\text{free}} \gg D_{\text{bound}}$, the approximation

$$\langle D\rangle \sim x_{\text{free}} D_{\text{free}} \quad (3)$$

holds if the molecules spend at least some time unbound, of the order of a few percent of the total.

This hypothesis is reasonable for the case when a narrow ^{1}H NMR spectrum is observed, as this testifies of a fast equilibrium between the two states.

The left-hand side panels of Figs. 1 and 2 demonstrate that the chosen molecules are hardly distinguishable according to their mobility along the gradient direction.

The right-hand side graphs in Figs. 1 and 2 can be interpreted, to first order, as a reflex of the time spent by a given molecule in a free state. Note that this is a rather crude approximation of the real dynamics ongoing in a porous solid material, where other factors affect the overall mobility. Abundant literature exists on this issue [7,8], and a more complete description of this dynamics in the framework of Chromatographic NMR will be the object of another study.

In the present work, the conditions of fast equilibrium were always assured, and monoexponential decay was a good approximation of the intensity decay of the stimulated echo. The measured mobility in the HRMAS–DOSY is the one in the direction of the applied gradient, in the specific, along the spinning axis. However, as a powder silica gel was used, this value should be representative of the general diffusion behavior.

The units of the diffusion coefficient used here are m^2/s, but the large variations observed for this parameter in our setup (up to four order of magnitudes) demands the use of a log scale. In the following, we shall refer directly to the log scale units only.

The first series of homologues discussed below is an aromatic set, benzene, naphthalene and anthracene (Fig. 1). We have analyzed this group of molecules before by Chr NMR, with excellent separation properties achieved by bare silica using water/acetonitrile as the solvent. The quality of the separation achieved by a $SiO_2/CDCl_3$ system is equivalent to the one observed under the previous conditions. The measured translational diffusion reflects the strength of their interaction with the solid adsorbent. In the case of aromatic compounds, π electrons are known to be the source of the interaction with the silica gel, and so the residence time on the silica gel is

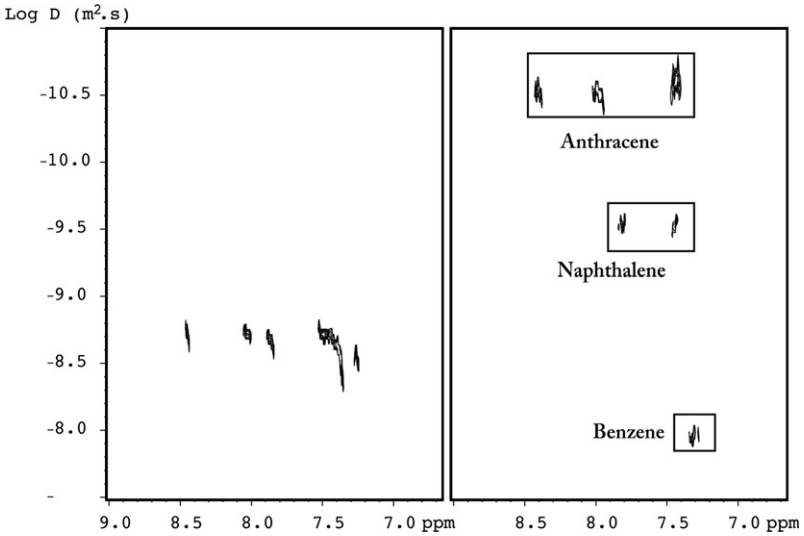

Fig. 1. (Left) DOSY plot of a mixture of aromatic homologues dissolved in $CDCl_3$ and (right) HRMAS–DOSY of the same mixture with the addition of bare silica gel.

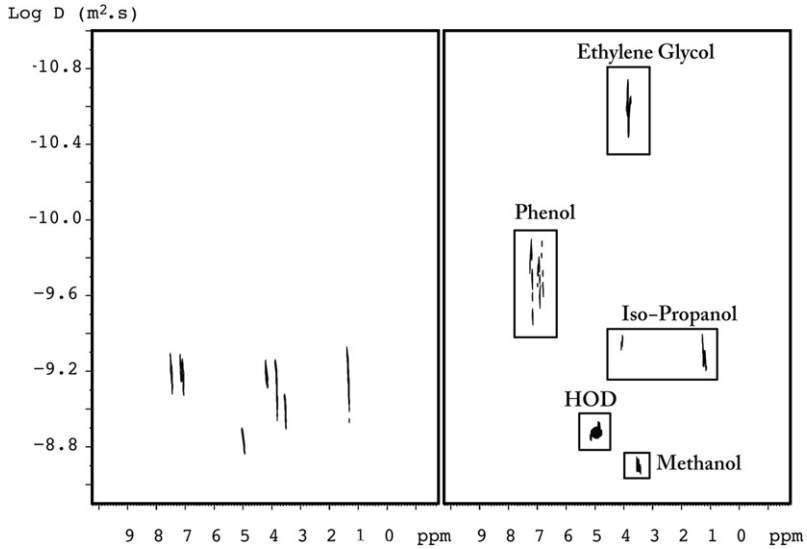

Fig. 2. (Left) DOSY plot of a mixture of alcohols in D_2O and (right) HRMAS–DOSY of the same mixture with the addition of bare silica gel.

expected to increase with the number of aromatic rings. Thus, anthracene is expected and observed to have the slowest translational rates ($\log(D) = -10.7$ m^2/s), while naphthalene has an apparent diffusion constant corresponding to $\log(D) = -9.5$ m^2/s and benzene to $\log(D) = -8.0$ m^2/s. In the absence of the silica gel, all three compounds have similar apparent diffusion coefficients, measured at $\log(D) = -8.5$ to -8.75 m^2/s. Note that benzene is apparently moving faster in the presence of the solid than in pure solution. This apparent contradiction is due to the contribution of gas phase molecules to the overall equilibrium. Direct evidence of this effect will be described in a successive article.

The HRMAS–DOSY spectra of a pure mix of *i*-PrOH, phenol, methanol and ethyleneglycol in D_2O and of the same mixture in the presence of SiO_2 are shown in Fig. 2a and b, respectively. The quality of the spectral separation induced by the silica gel is very good. In the pure liquid experiment, all signals, with the exception of MeOH and HDO, correlate with essentially the same coefficient diffusion, at about $\log(D) = -9.2$ m^2/s. MeOH is somewhat faster ($\log(D) = -9.0$ m^2/s), as expected on the basis of its smaller dynamic ratio, and for water the usual value of $\log(D) = -8.8$ m^2/s is observed. Upon addition of the silica gel, conversely, ethylene glycol understandably shows the largest relative delay, with a 1.4 log unit slower average self-diffusion coefficient. Phenol is the second slowest molecule, with $\log(D) = -9.7$ m^2/s. Note that this molecule is diffusing considerably slower than benzene, which suggests the OH function being responsible for most of its interaction with the solid phase. Isopropanol is next on the scale of progressively smaller measured apparent diffusion coefficients, with a moderate delay with respect to the pure liquid phase ($\Delta\log(D) = -0.2$ m^2/s). Surprisingly, HDO does not show a relevant shift in mobility upon addition of silica. This result could be the combination of two effects: (1) water being highly concentrated, the bound fraction would be giving a reduced overall contribution to the observed mobility and (2) a compensation between the solid-induced delay with the apparent speed-up observed for molecules with a non-negligible contribution to their translational diffusion coming from vapor phase, similarly to the case of benzene discussed earlier. This acceleration is all the way more evident in the case of low-boiling point methanol, whose apparent diffusion coefficient increases ($\Delta\log(D) = 0.3$ m^2/s) when the silica phase is added to the solution.

The two example test mixtures analyzed here demonstrated clearly the possibility of using Chr NMR with typical NMR solvents, with results completely equivalent to those obtained with well-established mobile phases of use in LC. This result supports further the hypothesis that in the particular setup of Chr NMR used here (namely a large solid/volume ratio), the solid phase is being the main factor in determining the equilibrium properties.

3. Conclusions

The possibility of using routine deuterated solvents in solid-assisted PFG HRMAS NMR analysis of mixtures (Chromatographic NMR) has been demonstrated. This is further evidence that the efficiency of the spectral separation in Chromatographic NMR relies mainly on the molecular affinity towards the solid, as previous experiments hinted to. This work tackled just one example of hydrophilic and hydrophobic molecules mixtures, which is far from providing an exhaustive list of possible compounds that can successfully undergo this type of analysis. The results of the current work represent an appreciable advantage in terms of applicability of Chromatographic NMR, as this can be applied on preparations very close to those routinely submitted to NMR analysis, by simple addition of an adapted adsorbent. Ongoing studies have been designed to further clarify the thermodynamic aspects of the equilibrium represented by the measured apparent self-diffusion coefficients and would be the object of a companion publication.

4. Experimental section

4.1. Samples and materials

The chromatographic phase used was LiChrospher®100Si (5 μm), obtained from Merck. All chemicals were purchased from Sigma–Aldrich and used as such, while the deuterated solvents were obtained from Eurisotop. The two test mixtures discussed in this paper were prepared as: benzene (5 mg mL^{-1}), naphthalene (1 mg mL^{-1}), anthracene (1 mg mL^{-1}) dissolved in $CDCl_3$ (mixture A) and phenol (25 mg mL^{-1}), methanol (25 μL mL^{-1}), iso-propanol (25 μL mL^{-1}), ethylene glycol (25 μL mL^{-1}) dissolved in D_2O (mixture B).

4.2. NMR measurements

All NMR experiments were performed at 400 MHz on a BRUKER Avance spectrometer equipped with a ^{1}H HRMAS probe

head producing magic-angle gradients with a maximum strength of 60 G cm^{-1}. Spectra were recorded at a spinning rate of 4000 Hz, at 303 and 298 K for mixtures A and B, respectively. All experiments were recorded using 4 mm o.d. zirconia rotors. Liquid diffusion measurements were performed with 12 μL rotors, while 50 μL rotors were used for the samples holding solution and silica gel. 16 mg of silica and 5 μL of solution were used for both mixtures.

The pulse sequence used was based on the stimulated echo and incorporated bipolar gradient pulses and a longitudinal Eddy current delay (BPP–LED). The shape of all gradient pulses was sinusoidal and the LED was held constant at 5 ms. The 2D diagrams were realized with gradient pulses of 2000 μs and a diffusion time of 400 ms. The gradient strength was logarithmically incremented in 32 steps from 2% to 95% of its maximum value, and 128 scans and 16 scans were recorded for mixtures A and B, respectively. After Fourier transformation and baseline correction, the diffusion dimension of the 2D DOSY maps was processed by means of the Bruker Xwinnmr software package (version 3.5). These maps show in the diffusion dimension a series of Gaussian peaks centered on the diffusion coefficient values, whose widths are proportional to the standard deviations of the respective *D* estimates achieved through mono- or multi-exponential fitting of the experimental data.

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